

## 6 THE KAHN UNIVERSAL SEROLOGICAL REACTION IN LEPROSY

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Kahn presents in his recent book (1) details of the latest of his contributions to serology, the concept that serological reactions with lipid antigens are phenomena common to the blood sera of all humans and animals and show distinctive patterns in health and disease. According to his method, by determining the titer of any serum with a lipid antigen in varying concentrations of saline and with different periods of ice-box incubation, reactions will be demonstrated in every instance in some portion of the pattern thus obtained. He reports the various patterns which these universal reactions show in normal persons and in a few diseases such as syphilis, yaws, leprosy, tuberculosis and malaria. He is of the opinion that in lepromatous leprosy the serological pattern of the universal reaction is distinctive, entirely different from the patterns obtained in either syphilis or yaws. While Kahn emphasizes the fact that his studies are preliminary, and that clinical interpretation both in health and in disease must be guarded until further investigations have been made, he feels that this test should permit differentiation of lepromatous and other forms of leprosy and that it offers a serologic indicator of activity of the disease. He has also expressed these views in a more recent publication (2).

During the year 1947, Kahn's universal serological test was performed in our laboratories on 130 cases of leprosy, parallel with 25 nonleprosy persons. These tests are the basis of this report.

### MATERIALS AND METHODS

The technique of the universal serologic test as performed in 1947 consisted of nine quantitative set-ups of ten tubes each. Each quantitative set-up was made with a given concentration of NaCl solution. The following nine concentrations were employed: 0.0, 0.15, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 per cent NaCl solutions. With each of these solutions, the serum

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was serially diluted from 1:1 to 1:640.<sup>2</sup> Each serum dilution was then mixed with the Kahn antigen suspension in a ratio of 6:1.<sup>3</sup> The tubes were shaken for 3 minutes, and then there was added 0.5 cc. of salt solution of the same concentration as used in preparing the serial dilution of the serum. The results were read immediately and also after standing for 4 and 24 hours in the ice-box. The ice-box temperature was maintained at 4°-5°C.

For the purpose of interrelating the degree of precipitation in the different quantitative set-ups with the salt concentrations, the precipitation results are divided, according to Kahn, into Zone I, of low salt concentration (0, 0.15, 0.3% NaCl); the central Zone II, of moderate salt concentration (0.6, 0.9, 1.2%); and Zone III, of high salt concentration (1.5, 1.8, 2.1%).

The central quantitative set-up (Zone II) is equivalent to a quantitative Kahn test when the results are read after the three-minute shaking period and without incubation. When precipitation does not reach the central zone, the universal reaction does not include a positive serodiagnostic reaction.

Graphs according to Kahn (<sup>1</sup>) are used throughout, because of the ease with which the universal reactions can be visualized in this form. Each graph consists of three sections based on the three readings of the precipitation results. In each section, the abscissa represents different salt concentrations (from 0 to 2.1%). The ordinate represents serial dilutions of serum with different salt concentrations (from 1:1 to 1:640). The reading scale is the same as in the Kahn standard test.

Sera were obtained from 130 patients, of which 20 were of the tuberculoïd type and 110 of the lepromatous type, the latter of which predominates in this hospital. In 1951, after an interval of four years, tests were repeated on 10 of the 110 lepromatous cases. The control material consisted of sera from 20 employees, nonleprous, and 5 cases of known syphilis.

## RESULTS

### NEGATIVE SERODIAGNOSTIC REACTIONS

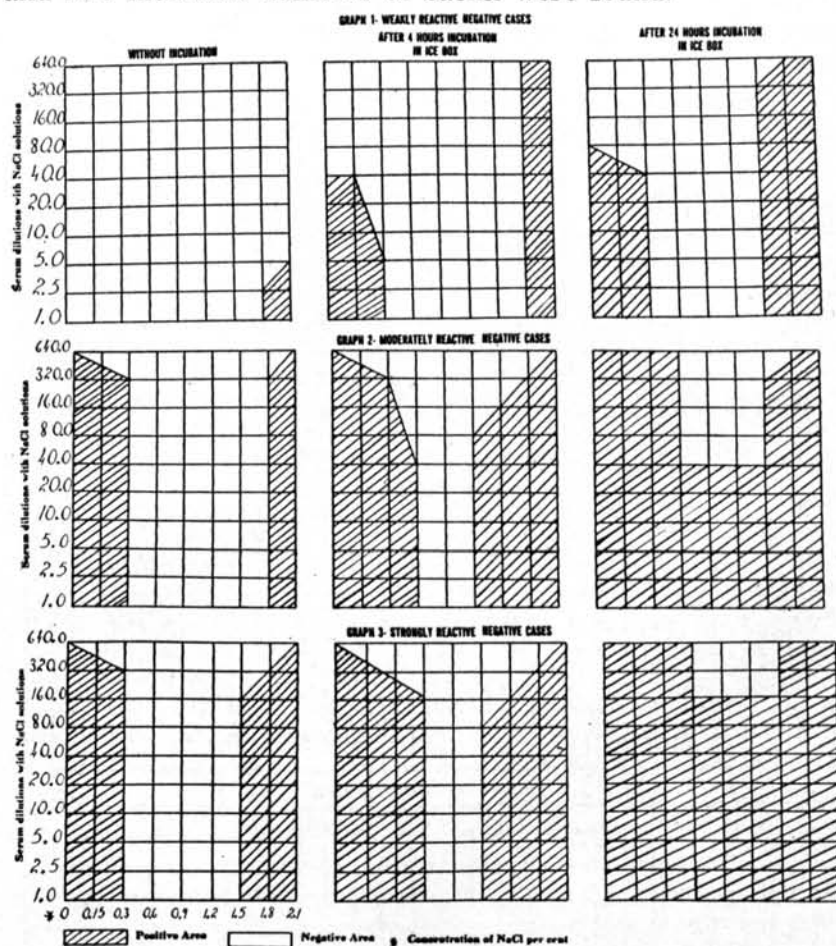
Text-fig. 1 presents three graphs showing universal reactions which indicate negative serodiagnostic results, or normal patterns. Although they were variable with each individual, patterns of this type were given by 49 of the leprosy cases, of which 34 were lepromatous and 15 tuberculoïd. Of the 49 cases, 32 gave weak reactions, 12 moderate reactions, and 5 strong reactions. It will be noted that precipitation did not reach the central quantitative set-up zone when the readings were made after the three-minute shaking period and before cold incubation. These types of serological patterns do not indicate a positive serodiagnostic reaction. They are common to normal in-

<sup>2</sup> The number of quantitative set-ups has recently been reduced from nine to seven. The serial dilution range is now 1:1 to 1:256, using seven tubes instead of ten.

<sup>3</sup> The Kahn standard antigen used was supplied by the Venereal Disease Research Laboratory, Communicable Disease Center, Atlanta, Ga.

dividuals, and are similar to the serological patterns of 20 of the 25 nonleprous persons whose sera we used as controls.

No characteristic serological pattern was correlated with the bacteriological status of this group. Of the 49 patients, 15 were arrested cases, 25 showed few bacilli in skin scrapings, and in 9 moderate numbers of bacilli were found.



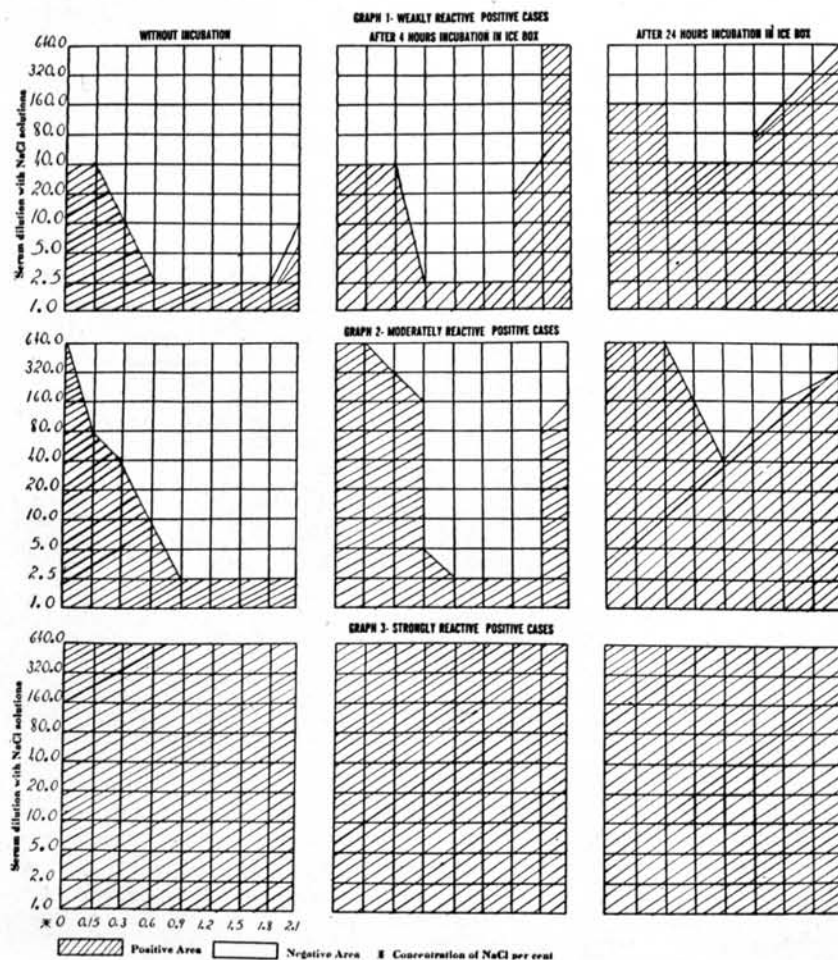
TEXT-FIG. 1. Representative graphs indicating negative serodiagnostic, or normal, results—with special reference to the central zone before ice-box incubation—with different degrees of the universal reaction. Graph 1, top row, weakly reactive negative sera (32 cases). Graph 2, middle row, moderately reactive negative sera (12 cases). Graph 3, bottom row, strongly reactive negative sera (5 cases).

Eighteen of the 49 cases had given positive standard Kahn, Kolmer and Mazzini reactions prior to sulfone therapy, but were serologically negative at the time this work was being done.



## POSITIVE SERODIAGNOSTIC REACTIONS

Text-fig. 2 presents three graphs showing the universal reactions which indicate positive serodiagnostic results. Patterns within this range were given by 71 of the leprosy cases, of which 66 were lepromatous and 5 tuberculoid. Of the 71 cases, 17 gave weakly positive reactions, 29 moderate positive reactions, and 25 strongly positive reactions. It will be noted that precipitation took place in the central quantitative set-up when the readings were made immediately after the three-minute shaking period. In the 25 cases showing strongly posi-



TEXT-FIG. 2. Representative positive serodiagnostic patterns. Graph 1, top row, weakly reactive positive sera (17 cases). Graph 2, middle row, moderately reactive positive sera (29 cases). Graph 3, bottom row, strongly reactive positive sera (25 cases).

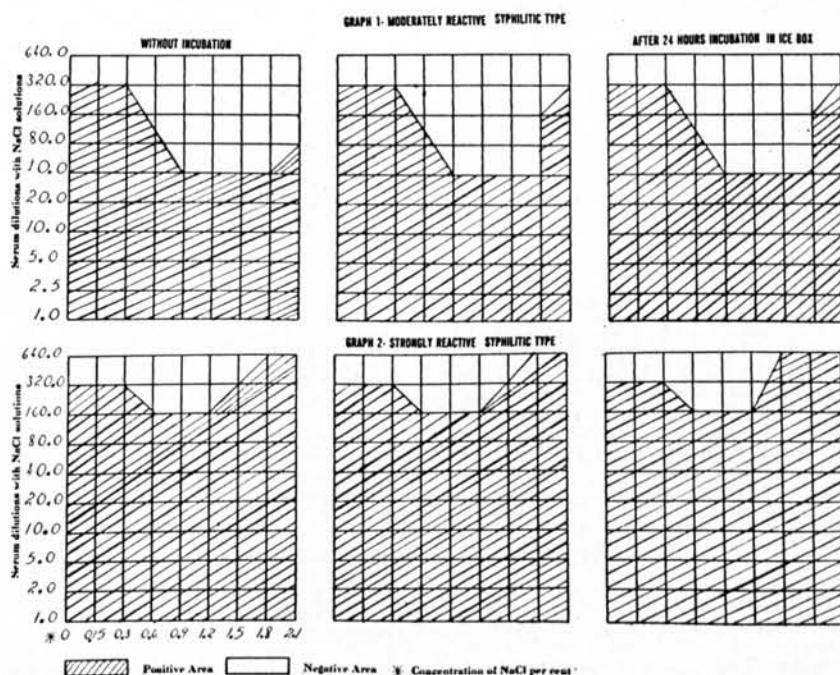
tive reactions, there was complete precipitation in all tubes at the time of the first reading, and this persisted thereafter.

The bacteriological status of this group was as follows: 1 case was arrested, 19 showed few bacilli, 11 showed moderate numbers of bacilli, and in 40 the bacilli in the skin scrapings were consistently numerous.

#### PATTERNS OF SYPHILIS

The universal reactions represented in Text-fig. 3 are serologic patterns that are characteristic of syphilis. Precipitation is seen immediately after the three-minute shaking period, and there is relatively little increase in precipitation on cold incubation for 4 and 24 hours. This holds true especially in the central zone. This serological pattern was shown in 10 cases of lepromatous leprosy which, as far as we are aware, are free of syphilis, as well as in the 5 known cases of syphilis whose sera were used as controls. Four of the 10 cases gave moderate positive reactions, and 6 of them strong reactions.

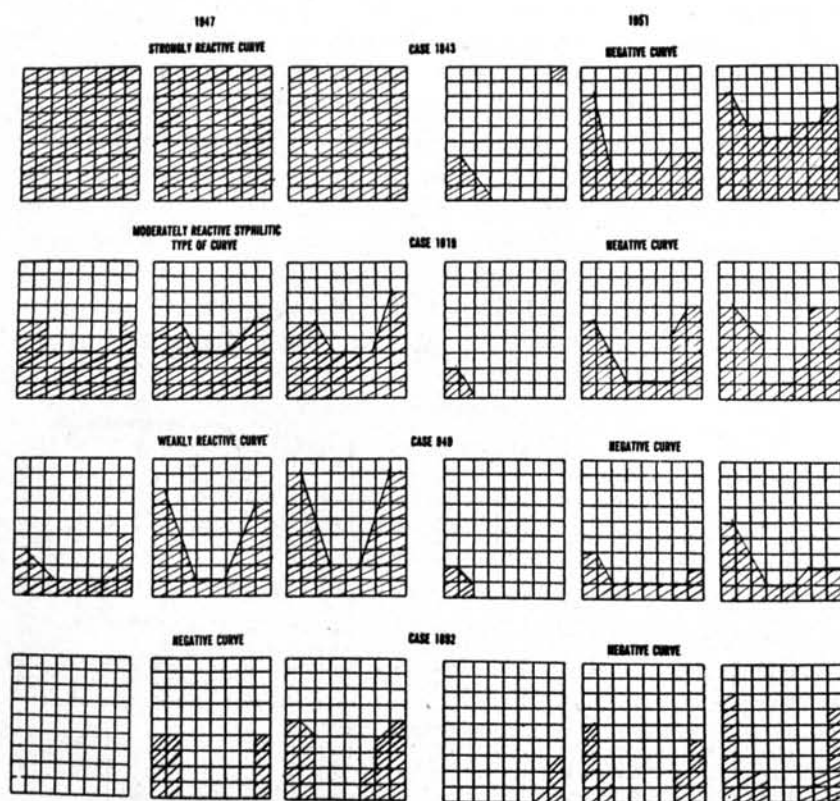
The bacteriological status of the 10 cases was: 1 case was arrested, 1 had few bacilli in skin scrapings, 3 moderate numbers of bacilli, and 5 numerous bacilli.



TEXT-FIG. 3. Patterns characteristic of syphilis, observed in lepromatous cases. Graph 1, top row, moderate syphilitic type of reaction (4 cases). Graph 2, bottom row, strong type of reaction (6 cases).

## CHANGES UNDER SULFONE THERAPY

Text-fig. 4 presents the serological patterns of 4 of the 10 patients who had had sulfone therapy for a period of four years when retested in 1951. On correlating the precipitation results with clinical and bacteriological findings, this group has shown marked improvement following therapy. This is likewise true of the remaining 6 patients whose graphs have not been included.



TEXT-FIG. 4. Changes in the serological patterns of four patients who had been under sulfone treatment for four years. The three diagrams at the left of each row are as of 1947, the three at the right are as of 1951.

## SUMMARY AND CONCLUSIONS

The universal serological reaction of Kahn was applied to the sera of 130 leprosy patients, of which 20 were of the tuberculoid type and 110 of the lepromatous type. The tests were repeated after an interval of 4 years on 10 of the 110 lepromatous cases. Controls consisting of sera from 20 employees

of this institution and 5 known cases of syphilis were run in parallel with the patients' sera.

A normal pattern, serodiagnostically negative, was observed in 49 cases. In 15 of them the disease was inactive, while in 35 it was active. Of those cases in which the disease was inactive, 13 were of the tuberculoid type and two of the lepromatous type.

A positive serological pattern was observed in 71 cases, of which 5 were tuberculoid and 66 lepromatous. The disease was active in 70 of these cases, and inactive in 1 case, which was of the tuberculoid type.

A positive serological pattern of the syphilitic type was observed in 10 lepromatous cases, in 9 of which the disease was active, in 1 inactive.

The serological patterns of 10 patients, rechecked after four years of sulfone therapy, showed reductions in the precipitation patterns in 9 instances, while in 1 case there was little change.

A considerable amount of precipitation is noted in the quantitative set-ups of high salt concentration after ice-box incubation at 4°-5°C. for 4 and 24 hours.

None of the sera in the present study showed a complete lack of precipitation in this universal serological test.

Twenty-five of the cases showed complete precipitation in all tubes after the three-minute shaking period and this persisted after ice-box incubation.

From our results it is concluded that there is no indication of any distinctive serologic pattern in lepromatous leprosy. Although the test is time-consuming, it is felt that serum reactors in leprosy are biologically significant, and that yearly checking of the universal serological test may be of some prognostic value. Our results, although meager, indicate that treatment can alter the type of the pattern in lepromatous leprosy.

#### RESÚMEN

La prueba serológica universal de Kahn, en su forma original que envuelve el uso de 90 tubos por prueba, se aplicó a sueros de 130 leprosos, 110 del tipo lepromatoso y 20 tuberculoides. Sueros testigos de 20 personas normales y 5 casos con sífilis fueron también sometidos a la prueba.

El tipo de patrón normal, serodiagnóstico negativo, se observó en 49 casos de lepra. El patrón serodiagnóstico positivo se observó en 71 casos. El patrón de tipo sífilítico se observó en 10 casos lepromatosos, ninguno de los cuales tenía sífilis. No hubo correlación ni bacteriológica ni con el estado de la enfermedad. Se concluye que no hay patrón

distintivo serológico en la lepra lepromatosa, pero que la prueba puede ser de valor pronóstico.

#### REFERENCES

1. KAHN, REUBEN L. Serology with Lipid Antigens. Baltimore: The Williams & Wilkins Co., 1950.
2. KAHN, R. L. Universal serologic reaction in leprosy. Ann. New York Acad. Sci. **54** (1951) 40-47.